# Temperature Dependent Development of *Lygus hesperus* (Hemiptera: Miridae) Nymphs

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**ABSTRACT**  *Lygus hesperus* Knight (Hemiptera: Miridae) is a key pest of fruit and vegetable crops, forages, and cotton (*Gossypium* spp.) in the western United States. Accurate models describing relationships between temperature and *L. hesperus* development are critical to the study of seasonal *L. hesperus* population dynamics. Development of *L. hesperus* nymphs was assessed at nine constant temperatures from 10 to 37.8°C. The relationships between temperature and development for each *L. hesperus* instar, and for the entire nymphal stage, were best described by six-parameter biophysical models indicating both low- and high-temperature inhibition of development. Development rates asymptotically approached zero with decreasing temperature in the lower thermal range, and decreased with increasing temperatures above 32.2°C. Nymphs did not survive from egg hatch to adulthood at either 10 or 37.8°C, and nymph mortality was >90% at both 12.8 and 35.0°C. The fifth instar exhibited the longest stadium, whereas the shortest stadia were associated with the second and third instars. Development rates of males and females did not differ, and the ratio of males to females was not different from 1:1 at any temperature. Our temperature-dependent development rate models for *L. hesperus* nymphs will facilitate control of insect physiological age in controlled laboratory experiments, and should be useful in planning and interpreting field studies on *L. hesperus* population dynamics.

**KEY WORDS**  biophysical model, temperature-dependent development, western tarnished plant bug, degree-day model

*Lygus hesperus* Knight (Hemiptera: Miridae) is a polyphagous agricultural pest in the western United States. *L. hesperus* feeding causes abscission of floral buds or deformation of developing fruit, which can lead to economic losses in fruit and vegetable crops and cotton (*Gossypium* spp.). Seasonal movements of *Lygus* among crop and noncrop hosts complicate efforts to manage *L. hesperus* populations, and accentuate the need for implementation of landscape-level management strategies (Carrière et al. 2006, Goodell 2009). Critical to the development and implementation of landscape-level management strategies is improved knowledge of seasonal ecology and population dynamics.

Insect population growth is regulated in part by relationships between temperature and insect development rates. Published studies of temperature-dependent *L. hesperus* nymph development reported development rates estimated from linear regression models (Champlain and Butler 1967, Butler and Wardecker 1971). Linear models also have been used to describe the relationship between temperature and development of other economically important *Lygus* pest spp. including *L. elisus* Van Duzee (syn. *L. desertus* Knight) and *L. lineolaris* (Palisot de Beauvois) (Butler 1970, Fleischer and Gaylor 1988, Bommireddy et al. 2004). Linear regression models often are useful for describing relationships between temperature and insect ontogeny within an intermediate thermal range for development. However, insect development rates typically become nonlinear near the low or high thermal limits for development. Therefore, linear regression models can over- or underestimate insect development rates at temperatures where low- or high-temperature inhibition occurs.

An alternative to linear models for describing relationships between temperature and insect development rates is the biophysical model developed by Sharpe and DeMichele (1977) and modified by Schoolfield et al. (1981). The biophysical model for insect development is based on biophysical laws and enzyme kinetics and is capable of describing temperature-dependent development rates over a range of temperatures where low- or high-temperature inhibition is encountered (Sharpe and DeMichele 1977, Schoolfield et al. 1981, Wagner et al. 1984). The pri-

mary objective of this study was to model relationships between temperature and *L. hesperus* nymph development by using the biophysical model to assess low- or high-temperature inhibition of development.

## Materials and Methods

***L. hesperus* Nymph Development.** A laboratory colony of *L. hesperus* was started from adults collected from alfalfa fields (*Medicago sativa* L.), and was maintained on green bean pods (*Phaseolus vulgaris* L.) and raw sunflower seeds (*Helianthus annuus* L.) at ≈27°C with a photoperiod of 14:10 (L:D) h. Insects used for development assays were ≤3 generations removed from field populations. Fifteen whole green bean pods were placed into each of two colony cages (Bioquip Products, Rancho Dominguez, CA, model 1450BS) containing ≈500 *L. hesperus* adults per cage. Beans were removed from adult cages after 24 h and examined for the presence of eggs. Each bean with eggs was cut into half and each half was placed on moist filter paper in a 100- by 15-mm petri dish (Fisher, Pittsburgh, PA). Petri dishes were sealed with Parafilm M (Pechiney Plastic Packaging Company, Chicago, IL) to prevent beans from desiccating. Petri dishes were monitored twice daily for the presence of newly eclosed nymphs beginning on the fifth day after oviposition. Each neonate was transferred to an 18.5-ml clear plastic vial (Thornton Plastics, Salt Lake City, UT). Each vial was provisioned with a section of a green bean pod with the cut ends sealed with paraffin wax and was closed with a ventilated lid.

Development times of nymphs were determined at nine constant temperatures: 10, 12.8, 15.6, 21.1, 26.7, 29.4, 32.2, 35, and 37.8°C (±1°C). Constant temperatures were maintained in environmental chambers (Percival Scientific Inc., Perry, IA, model I30BLL) with 50–70% RH and a photoperiod of 14:10 (L:D) h. Temperature and relative humidity within each chamber were monitored using HOBO Data Loggers (Onset Computer Corporation, Pocesset, MA). Nymphs ($n = 20$/temperature, $N = 180$) were assigned randomly to temperatures on the day of eclosion. Preliminary assays indicated nearly 100% mortality of first instars at 10 and 37.8°C. Therefore, separate sets of nymphs were reared at 26.7°C, then transferred to 10 or 37.8°C within 8 h of the beginning of the second, third, fourth, or fifth stadium ($n = 10$ of each instar per temperature). Vials were examined every 24 h for mortality or for cast exoskeletons signifying molting. Cast exoskeletons were removed immediately from the vials. Gender was determined for each insect that survived to adulthood. Green bean pods were replaced each Monday, Wednesday, and Friday in vials containing third, fourth, or fifth instars. To reduce accidental mortality from handling early instars, beans were only replaced once weekly in vials containing first or second instars. Assays were conducted three independent times (trial), with each trial using a different cohort of nymphs, and temperatures were assigned randomly to environmental chambers before each trial.

**Statistical Analyses.** Separate analyses were used to model the temperature-dependent development of each *L. hesperus* instar and for complete nymph development from egg hatch to adulthood. Development times (days) at each constant temperature from 10 to 37.8°C were used to calculate development rates for use as inputs in the development rate model of Sharpe and DeMichele (1977), as modified by Schoolfield et al. (1981). The general form of the model is

$$r(T) = \frac{RHO25 \dfrac{(T + 273.15)}{298.15} \exp\left[\dfrac{HA}{R}\left(\dfrac{1}{298.15} - \dfrac{1}{(T + 273.15)}\right)\right]}{1 + \exp\left[\dfrac{HL}{R}\left(\dfrac{1}{TL} - \dfrac{1}{(T + 273.15)}\right)\right] + \exp\left[\dfrac{HH}{R}\left(\dfrac{1}{TH} - \dfrac{1}{(T + 273.15)}\right)\right]}$$

where $r(T)$ = development rate at temperature $T$ (°C), $RHO25$ = development rate at 25°C with no enzyme inhibition, $HA$ = enthalpy of activation of the reaction catalyzed by a rate-controlling enzyme, $TL$ = Kelvin temperature at which the rate-controlling enzyme is half inactive because of low-temperature inhibition, $HL$ = change in enthalpy associated with low-temperature inhibition of the rate controlling enzyme, $TH$ = Kelvin temperature at which the rate-controlling enzyme is half inactive because of high-temperature inhibition, $HH$ = change in enthalpy associated with high-temperature inhibition of the enzyme, and $R$ = the universal gas constant (1.987 cal °K$^{-1}$mol$^{-1}$). $RHO25$ and $HA$ are the most influential model parameters at intermediate temperatures, $TL$ and $HL$ dominate at low temperatures when low-temperature inhibition occurs, and $TH$ and $HH$ dominate at high temperatures in the presence of high-temperature inhibition. Models were fit to the data by using the SAS program of Wagner et al. (1984) after modifying the syntax for compatibility with SAS version 9.2 (SAS Institute, Cary, NC). The SAS program developed by Wagner et al. (1984) determines the number of parameters (two, four, or six) to be used in the nonlinear model for a given data set, selects starting values of these parameters, and computes least-squares estimates of the parameters by using Marquardt techniques. A best fit regression to a two-, four-, or six-parameter model indicates no temperature inhibition, low- or high-temperature inhibition, or low- and high-temperature inhibition, respectively.

In separate analyses, temperature-dependence of stadium lengths was compared among instars and between genders by using the GLIMMIX procedure of SAS. Insect instar or gender, temperature, and their interactions were the fixed effects and stadium length (d) was the dependent variable. Trial was included as a random variable. The SLICEDIFF and ADJUST = SIMULATE options of the LSMEANS statement were used to analyze differences among simple effects when significant main effect interactions were observed. The influence of temperature on gender of bugs surviving to the adult stage was examined using logistic regression (PROC GLIMMIX) with temperature as the fixed effect, female as the response variable, and trial as the random variable.
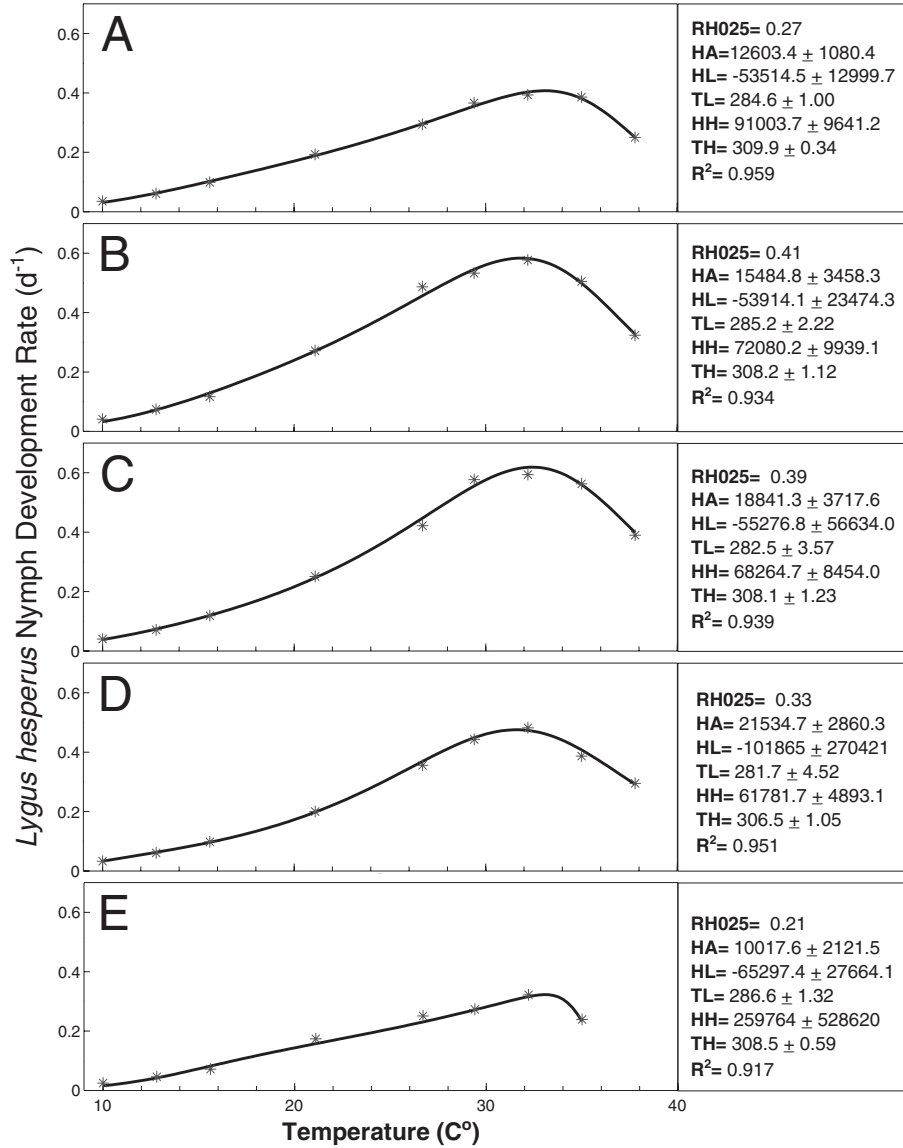
**Fig. 1.** Development rate plots and model parameters from six-parameter biophysical models for temperature-dependent development of first (A), second (B), third (C), fourth (D), and fifth (E) instar *L. hesperus*. Data points represent observed mean development rates at constant temperatures of 10, 12.8, 15.6, 21.1, 26.7, 29.4, 32.2, 35, and 37.8°C.

### Results and Discussion

Temperature-dependent development of each *L. hesperus* instar (Fig. 1A–E) and the complete nymph stage (Fig. 2) were best described by six-parameter biophysical models indicating both low- and high-temperature inhibition of nymph development. Model predicted development times (d) at each temperature generally deviated <10% from observed development times (Table 1). The overall relationships between temperature and development rates were similar for each instar (Fig. 1A–E). The highest development rates were observed at 32.2°C, and development rates decreased at higher temperatures (Figs.

1, 2). At the lower end of the observed thermal range, development rates asymptotically approached zero with decreasing temperature (Figs. 1 and 2).

Previous reports of *L. hesperus* nymph development used linear regression to model observed development rates (Champlain and Butler 1967, Butler and Wardecker 1971). However, Champlain and Butler (1967) did not include nymph development rates at 10°C in their model because the observed development rate at that temperature was not close to the prediction from the linear regression. Compared with the previously published temperature-dependent development models for *L. hesperus* nymphs, our devel-
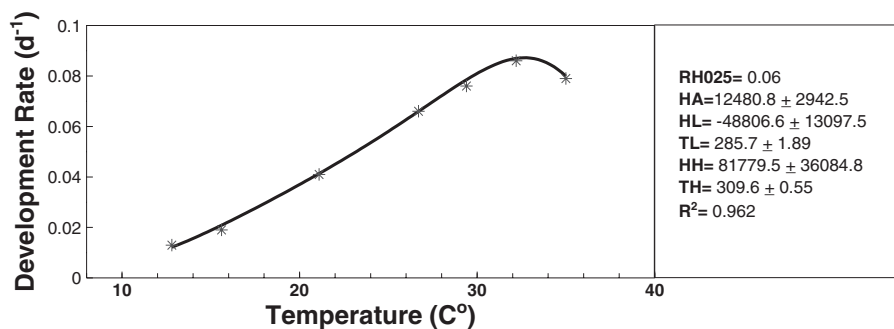
**Fig. 2.** Development rate plot and model parameters from a six-parameter biophysical model for temperature-dependent development of *L. hesperus* nymphs from egg hatch to adulthood. Data points represent observed mean development rates at constant temperatures of 12.8, 15.6, 21.1, 26.7, 29.4, 32.2, and 35°C.

opment rate models provide useful predictions of nymph development rates over a broader range of temperatures. In light of our results, the biophysical model might also have utility for describing temperature-dependence of other processes in *L. hesperus* biology such as development of eggs or adult reproductive organs.

Complete nymph development from egg hatch to adulthood was not observed for individual insects maintained at 10 or 37.8°C because of high mortality. Based on our observations of nymphs transferred from 26.7°C to either 10 or 37.8°C at the beginning of the second, third, fourth, or fifth stadium, development was observed for each instar at both 10 and 37.8°C except for fifth instars held at 37.8°C (Fig. 1A–E; Table 1). However, mortality typically occurred during or within 48 h of molting at these temperatures and none of the nymphs survived to a subsequent molt. Mortality also generally was high (>90%) for nymphs maintained at 12.8 and 35°C (Table 1). At these latter temperatures mortality also tended to occur during or within 48 h of molting as was observed at 10 and 37.8°C. High mortality at low and high temperature was reported previously for *L. hesperus* (Champlain and Butler 1967) and *L. elisus* (Bommireddy et al. 2004) nymphs molting to adults, and for hatching eggs of *L. lineolaris* (Ridgway and Gyrisco 1960). Potentially, *Lygus* nymphs are more susceptible to desiccation during ecdysis and at temperature extremes. Alternatively, mortality during ecdysis may result from physiological injury caused by prolonged exposure to low or high temperatures. Regardless, our results indicate *L. hesperus* nymphs survive and develop for nearly a month at a constant 10°C and for 3–4 d at a constant 37.8°C (Table 1).

Our results are in general agreement with previously reported lower developmental thresholds for use in calculating degree-day phenological models for *L. hesperus* nymphs (Champlain and Butler 1967, Sevacherian et al. 1977, Pickel et al. 1990). Based on linear regression of development rates, Champlain and Butler (1967) reported a lower developmental threshold of 8°C for *L. hesperus* nymphs. In contrast, Sevacherian et al. (1977) suggested a developmental threshold of 11.1°C based on published studies of *L.*

*hesperus* temperature dependent development. Based on field studies, Pickel et al. (1990) reported a lower developmental threshold of 12.2°C. Estimating lower developmental thresholds can be difficult and subjective because development rates asymptotically approach zero at the lower thermal limits for development. Although we observed nymph development at 10°C, development rates were $<0.01$ d$^{-1}$, and none of the nymphs introduced to this temperature as first instars survived beyond the second stadium. The greatly prolonged development time and the lack of survival to adulthood at this temperature suggests 10°C would be an appropriate lower developmental threshold for degree-day models predicting *L. hesperus* nymphal development.

Upper thermal thresholds for *L. hesperus* development have not been reported, but such thresholds are commonly employed when formulating degree-day models for other insects. Two techniques for estimating high temperature thresholds for degree-day calculations are the horizontal cut-off and the vertical cut-off methods (Baskerville and Emin 1969). The horizontal threshold cut-off is the temperature above which the insect development rate ceases to increase, which typically is lower than the physiological upper thermal limit for development. Therefore, the horizontal method assumes that development rates are constant at temperatures above the upper optimal temperature for development. The vertical threshold cut-off is the temperature above which the development rate equals zero. Thus, the vertical threshold cut-off assumes that development rate continues to increase, beyond the optimal temperature for development, until the cut-off threshold is exceeded. Neither threshold cut-off method adequately accommodates high-temperature inhibition of development. In practical use, the use of horizontal threshold cut-offs may provide more accurate predictions for insect development times compared with the use of vertical threshold cut-offs (Roltsch et al. 1999). Based on our results, the horizontal upper threshold cut-off for *L. hesperus* nymphs is ≈32°C (Fig. 2; Table 1). However, upper developmental thresholds should be used cautiously because temperatures within plant canopies where *Lygus* nymphs reside may be lower than day-

Table 1. Mean observed stadium lengths (d) ± SE, model predicted stadium lengths, and numbers of *Lygus hesperus* nymphs that molted at constant temperatures from 10 to 37.8°C

| Instar | Estimate type | Temperature (°C) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 10[a] | 12.8 | 15.6 | 21.1 | 26.7 | 29.4 | 32.2 | 35 | 37.8[a] |
| First | Observed | 28.8 ± 0.60 | 17.1 ± 0.42 | 10.4 ± 0.38 | 5.3 ± 0.38 | 3.4 ± 0.40 | 3.0 ± 0.40 | 2.7 ± 0.39 | 2.8 ± 0.42 | 3.9 ± 1.84 |
| | Predicted | 33.1 | 15.5 | 9.5 | 5.3 | 3.4 | 2.8 | 2.4 | 2.4 | 4.0 |
| | n | 12 | 36 | 51 | 54 | 44 | 43 | 49 | 36 | 2 |
| Second | Observed | 25.5 ± 0.51 | 15.1 ± 0.46 | 8.9 ± 0.39 | 3.9 ± 0.38 | 2.2 ± 0.41 | 2.1 ± 0.42 | 1.9 ± 0.39 | 2.1 ± 0.45 | 3.2 ± 0.60 |
| | Predicted | 30.1 | 13.8 | 7.7 | 3.7 | 2.2 | 1.8 | 1.7 | 2.0 | 3.1 |
| | n | 19 | 26 | 47 | 51 | 39 | 34 | 47 | 28 | 12 |
| Third | Observed | 26.1 ± 0.50 | 14.4 ± 0.50 | 8.8 ± 0.41 | 4.2 ± 0.39 | 2.4 ± 0.42 | 2.0 ± 0.44 | 1.9 ± 0.40 | 1.9 ± 0.50 | 2.6 ± 0.42 |
| | Predicted | 26.2 | 13.8 | 7.7 | 3.7 | 2.2 | 1.8 | 1.7 | 2.0 | 3.1 |
| | n | 17 | 20 | 40 | 48 | 37 | 32 | 42 | 20 | 18 |
| Fourth | Observed | 32.0 ± 0.45 | 15.7 ± 0.56 | 10.3 ± 0.43 | 5.2 ± 0.39 | 2.9 ± 0.42 | 2.4 ± 0.43 | 2.3 ± 0.43 | 2.7 ± 0.64 | 3.4 ± 0.67 |
| | Predicted | 30.4 | 15.9 | 10.4 | 5.1 | 2.7 | 2.2 | 2.1 | 2.5 | 3.4 |
| | n | 27 | 14 | 32 | 46 | 35 | 32 | 34 | 10 | 11 |
| Fifth | Observed | 41.2 ± 0.49 | 21.9 ± 0.80 | 14.0 ± 0.44 | 6.5 ± 0.41 | 4.1 ± 0.44 | 3.8 ± 0.44 | 3.0 ± 0.46 | 4.2 ± 0.95 | — |
| | Predicted | 62.5 | 23.5 | 12.1 | 6.3 | 4.4 | 3.7 | 3.2 | 4.3 | — |
| | n | 21 | 6 | 31 | 40 | 31 | 30 | 25 | 4 | 0 |
| Nymph Stage | Observed | — | 76.6 ± 3.68 | 51.6 ± 0.86 | 24.8 ± 0.38 | 15.2 ± 0.20 | 13.3 ± 0.27 | 11.7 ± 0.30 | 12.8 ± 0.67 | — |
| | Predicted | 0 | 82.3 | 47.8 | 24.2 | 15.3 | 12.7 | 11.5 | 12.5 | — |
| | n | 0 | 6 | 31 | 40 | 31 | 30 | 25 | 4 | 0 |

[a] Most insects died during or within 48 h of molting; therefore, development of each instar was assessed separately.

time ambient temperatures measured above the crop canopy.

Significant differences in stadium lengths were observed among instars ($F = 131.1$; d.f. = 4, 609; $P < 0.001$), but the instar by temperature interaction ($F = 23.4$; d.f. = 31, 1077; $P < 0.001$) also was significant. This significant interaction indicated that differences among instars in development times were conditional upon temperature. Stadium lengths differed significantly ($\alpha = 0.05$) among instars within each temperature except at 35 and 37.8°C, but the observed patterns in stadium lengths corresponding to the different instars were similar at each temperature (Table 1). At temperatures from 10 to 32.2°C, the fifth stadium was significantly longer than other stadia (Fig. 1; Table 1), which was consistent with the report by Butler and Wardecker (1971). In addition, the first and fourth stadia were significantly longer than the second and third stadia (Fig. 1; Table 1). This observation may be important because the fourth and fifth instars of *L. hesperus* may be more injurious to host plants than earlier instars (Zink and Rosenheim 2005, Cooper, unpublished data).

Gender did not significantly influence development time ($F = 0.07$; d.f. = 1, 152.2; $P = 0.785$), and there was no gender by temperature interaction ($F = 0.18$; d.f. = 5, 152.5; $P = 0.971$). Therefore, the effect of temperature ($F = 939.9$; d.f. = 5, 152.5; $P < 0.001$) on development time was similar for both genders. Our observed lack of a gender effect on nymph development time was consistent with reports by Butler and Wardecker (1971) on development of *L. hesperus* and by Bommireddy et al. (2004) on development of *L. elisus.* In addition, there were no differences among temperatures from 12.8 to 32.2°C in the gender ratios of insects surviving to adulthood ($F = 0.78$; d.f. = 4, 8; $P = 0.570$). Based on confidence limits of the estimated probabilities that surviving insects would be female, the ratio of male to female insects was not different from 1:1. Observations at 10, 35, and 37.8°C were excluded from the analysis of gender ratio because of high nymph mortality.

Our results clearly demonstrate low- and high-temperature inhibition of *L. hesperus* nymph development rates. Our temperature-dependent development rate models provide descriptions of the relationships between temperature and *L. hesperus* nymph development that are more reasonable, statistically and biologically, than those in earlier reports. These models will allow improved experimental control of *L. hesperus* physiological age in controlled laboratory experiments and may facilitate the design and interpretation of field studies of *L. hesperus* population dynamics.

## Acknowledgments

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